July 2022 | EN

virotype® BVDV 2.0 RT-PCR Kit Handbook

For detection of RNA from *Bovine Viral Diarrhea Virus* (BVDV)

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 112

REF 100 reactions (Cat. no. VT280385)

REF 500 reactions (Cat. No. VT280387)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

INDICAL
BIOSCIENCE

Contents

Kit contents 3
Intended use 3
Symbols 4
Quality control 4
Storage 5
Safety information 5
Introduction 6
Principle 7
RNA extraction 8
Equipment and reagents to be supplied by user 10
Important notes 11
General precautions 11
Protocol: Real-time RT-PCR for detection of RNA from *Bovine Virus Diarrhea Virus* 13
Important points before starting 13
Things to do before starting 13
Procedure 14
Data analysis and interpretation 16
Interpretation of results 16
Change index 24

Kit contents

virotype BVDV 2.0 RT-PCR Kit Cat. no. Number of reactions	(100) VT280385 100	(500) VT280387 500
Master Mix (tube with orange cap), includes primers, probes and enzymes	1 x 800 µl	5 x 800 µl
Positive Control (tube with red cap)	1 x 150 µl	2 x 150 µl
Negative Control (tube with blue cap)	1 x 150 µl	2 x 150 µl
Handbook	1	1

Intended use

The virotype BVDV 2.0 RT-PCR Kit is intended for the detection of RNA from *Bovine Viral Diarrhea Virus* (BVDV) in blood, plasma, serum, tissue, milk, and ear tissue samples (individual or pooled) from cattle.

The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 112) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

Legal manufacturer

Lot number

Use by date

Temperature limitations for storage

Handbook

Catalog number

Material number

Protect from light

For samples from cattle

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of virotype BVDV 2.0 RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the virotype BVDV 2.0 RT-PCR Kit should be stored at -30°C to -15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing (>3x), as this may reduce assay sensitivity. Freeze the components in aliquots if they will only be used intermittently.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under **compliance@indical.com**.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Introduction

The virotype BVDV 2.0 RT-PCR Kit is a sensitive solution for the detection of *Bovine Viral Diarrhea Virus* (BVDV) RNA in samples from cattle.

Bovine Viral Diarrhea/ Mucosal Disease (BVD/ MD) is one of the most important infectious diseases of cattle worldwide. The causative agent, *Bovine Viral Diarrhea Virus* (BVDV) is a single-stranded RNA virus which belongs to the genus *Pestivirus* and exists in the two distinct species *Pestivirus A* (BVDV-1) and *Pestivirus B* (BVDV-2). Further subtyping is possible. According to their growth in cell culture, BVDV isolates of both species are classified into the two biotypes cytopathic (cp) and non-cytopathic (ncp).

Depending on the immune status of the animals, BVDV infections may lead to gastro-intestinal and respiratory symptoms of different severity as well as to reproductive problems. These are caused by transplacental infection of the fetus leading to abortions, congenital malformations, and in case of infection before immunocompetence to persistently infected (PI or viremic) calves.

PI animals only emerge through prenatal infection, whereas postnatal infections lead to transient viremia, inducing the production of neutralizing antibodies. Mucosal Disease occurs when persistently viremic animals carry BVD viruses of both biotypes (cp and ncp BVDV).

Unidentified PI animals are responsible for the spread of BVDV as they excrete high doses of the virus during their whole life. Thus, they may infect pregnant animals which in turn may give birth to new PI animals. The major route to successfully combatting the disease is the early identification of those PI animals.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is identified using fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows detection of the accumulating product without the need to re-open the reaction tubes afterward.

The virotype BVDV 2.0 RT-PCR Kit contains all of the necessary reagents for the detection of BVDV RNA, including a Positive and Negative Control. With this kit, both reverse transcription and PCR are performed in one reaction tube, reducing the risk of contamination.

The kit contains two internal controls. The endogenous control (EC) detecting the β-actin gene present within the sample and the exogenous control (IC) permitting tests for successful extraction and amplification by adding it to the RNA purification procedure.

Both internal control systems exclude the possibility of false-negative results

The kit uses three specific primer/probe combinations:

- FAM™ fluorescence for RNA from BVDV
- HEX™ fluorescence for the endogenous control (EC)
- Cy®5 fluorescence for the exogenous internal control (IC)

A Positive Control serves to verify the functionality of the reaction mix for the amplification of the BVDV RNA target.

RNA extraction

The virotype BVDV 2.0 RT-PCR Kit is intended for the detection of BVDV RNA from blood, plasma, serum, tissue, milk and ear tissue samples from cattle.

Due to the high sensitivity of the test kit, individual or pooled samples can be tested. The pools can consist of up to 25 ear tissue samples or 50 individual blood samples (blood, plasma, serum), or 50 milk samples. An increase of pool sizes up to 100 individual samples is possible when using blood or milk.

Note: For use in Germany the specifications described in the „*Amtliche Methodensammlung*" apply.

Prior to real-time RT-PCR, viral RNA must be extracted from the starting material.
Optional: The exogenous internal control RNA (intype IC-RNA, Cat. no. IC289970) must be added to the **lysis buffer** prior the extraction procedure. In most cases, 2 µl intype IC-RNA per sample are suitable but should be tested in the extraction system used.

For rapid preparation of ear tissue samples (diameter 2 – 3 mm) without RNA isolation the use of virotype Tissue Lysis Reagent is recommended. Ear tissue lysates should be tested immediately and can be stored at 2 – 8°C for up to 12 h or at -20 to -80°C.

INDICAL offers a range of validated kits for the extraction of RNA from animal samples.

Extraction based on magnetic beads:

- **IndiMag® Pathogen Kit** (SP947457)
- **IndiMag Pathogen Kit w/o plastics** (SP947257)
- **IndiMag Pathogen IM48 Cartridge** (SP947654P608, SP947654P224)
- **IndiMag Pathogen KF96 Cartridge** (SP947855P196)

Extraction based on spin columns:

- **IndiSpin® Pathogen Kit** (SP54104, SP54106)
- **IndiSpin QIAcube® HT Pathogen Kit** (SP54161)

Fast Lysis

- **virotype Tissue Lysis Reagent** (SP289992, SP289993)

If real-time RT-PCR is not performed immediately after extraction, store the RNA at -20°C or at -80°C for longer storage.

For further information on automated and manual extraction of BVDV RNA from different sample types, refer to the respective handbook or contact INDICAL Support at **support@indical.com**.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- **Optional** (not included in the kit): intype IC-RNA (Cat. No. IC289970, INDICAL BIOSCIENCE)
- Pipets
- Nuclease-free, aerosol-resistant pipet tips with filters
- Nuclease-free (RNase/DNase-free) consumables. Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive identification of viral nucleic acids
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen real-time cycler
- Appropriate strip tubes and caps or 96-well optical microplate with optical sealing film or cover for chosen real-time cycler

Important notes

General precautions

The user should always pay attention to the following:

- Use nuclease-free pipet tips with filters.
- Store and extract positive materials (specimens, positive controls and amplicons) separately from all other reagents and add them to the reaction mix in a spatially separated facility.
- Thaw all components on ice before starting as assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Negative control

At least one negative control reaction should be included in each PCR run, containing all the components of the reaction except for the pathogen template. This enables assessment of contamination in the reaction.

Positive control

When performing PCR on unknown samples, it is recommended to perform a positive control reaction in the PCR run, containing a sample that is known to include the targeted viral RNA. A positive control serves to prove the functionality of the pathogen assay, e.g., the correct setup of the reaction mix. Use 5 µl of the Positive Control provided with

the virotype BVDV 2.0 RT-PCR Kit to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, two extraction and amplification control assays are included in the test kit.

An endogenous internal control (EC) detects the β-actin gene present within the sample, whereas the exogenous internal control (IC) detects intype IC-RNA, which must be added to the lysis buffer prior to the extraction procedure (optional application). The use of both control systems allows extraction and amplification to be monitored, also in samples that would show at least partial inhibition due to the sample quality. No exogenous internal control (IC-RNA) is required for fast lysis procedures.

Protocol: Real-time RT-PCR for detection of RNA from *Bovine Virus Diarrhea Virus*

Important points before starting

- Please read „Important notes“ on page 11 before starting.
- It is strongly recommended to use the intype IC-RNA to monitor extraction and amplification, as well as any partial inhibition. Please add the respective volume to the lysis buffer prior to extraction.
- Include at least one positive control (Positive Control) and one negative control (Negative Control) per PCR run.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- RNA is unstable. Perform the protocol without interruption.

Things to do before starting

- Thaw all reagents on ice and protect from light.
- Before use, spin the reagents briefly.
- Maintain reagents on ice or in a cooling block during PCR setup.

Procedure

1. Before use, mix the Master Mix by inverting 5 times or until mixed thoroughly, then centrifuge briefly to collect the fluids.
2. Pipet 8 µl of the Master Mix into each reaction tube. Then add 5 µl of the sample RNA (Table 1).

   Include positive and negative control reactions.

   Positive Control: Use 5 µl of the positive control (Positive Control) instead of sample RNA.

   Negative Control: Use 5 µl of the negative control (Negative Control) instead of sample RNA.

Table 1. Preparation of reaction mix

Component	Volume
Master Mix	8 µl
Sample	5 µl
Total volume	**13 µl**

3. Close the reaction tubes or seal the plate and invert 5 times or until mixed thoroughly. Then centrifuge briefly to collect the fluids.
4. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Pathogen/ internal control	**Reporter**
BVDV	FAM
Endogenous internal control (EC)	HEX/ JOE™[1]
Exogenous internal control (IC)	Cy5
Passive reference[2]	ROX™[2]

1 Use the option appropriate for your thermal cycler.
2 Internal reference for use with ABI PRISM® Sequence Detection Systems (Applied Biosystems®)

5. Run the real-time RT-PCR protocol according to Table 3.

Table 3. Real-time RT-PCR protocol for BVDV 2.0

Step	**Temperature**	**Time**	**Number of cycles**
Reverse Transcription	50°C	10 min	1
Initial Activation	95°C	2 min	1
2-step cycling			
Denaturation	95°C	5 s	40
Annealing/Extension*	60°C	30 s	

* Fluorescence data collection, approximate run time 64min (CFX96, Bio-Rad™)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the FAM, HEX and Cy5 channels with a C_T[1] < 35. The Negative Control must give no signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 4 on page 19.

The sample is positive for BVDV, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM channel (regardless of any signal in the HEX and/ or Cy5 channel).
- The Positive Control yields a signal in all channels.
- The Negative Control does not yield a signal in the FAM, HEX and Cy5 channel.

Note that very high concentrations of BVDV RNA in the sample may lead to a reduced signal or no signal for the internal controls (HEX for the EC, Cy5 for the IC) due to competition with the internal controls.

---

[1] Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence

The sample is negative for BVDV, and the assay is valid, if the following criteria are met:

- The sample **does not yield** any signal in the FAM channel.
- When used **with** intype IC-RNA: The sample yields a signal in the HEX and Cy5 channel.
- When used **without** intype IC-RNA: The sample yields a signal in the HEX channel.
- The Positive Control yields a signal in all channels.
- The Negative Control does not yield a signal in the FAM, HEX and Cy5 channel.

The sample results are inconclusive, and the assay is invalid, if the following criteria are met:

- The sample yields no signal in any of the channels.

If no signal is detected, the result is inconclusive. The absence of a signal for the endogenous internal control (EC) and the exogenous internal control (intype IC-RNA, IC, only when applied) indicate strong PCR inhibition and/ or other malfunctions, e.g., during extraction.

To check for inhibition, we recommend 1:5 dilution of the sample RNA in nuclease free water, to repeat the RNA extraction procedure, or repeat the whole test procedure starting with new sample material.

Check that there is a fluorescence signal in the FAM, HEX, and Cy5 channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to incorrect setup of the reaction mix or incorrect cycling conditions.

Additional information given by the endogenous and exogenous internal control systems:

The **lack of a Cy5 fluorescence signal** (applicable only if intype IC-RNA was applied) can be caused by insufficient sample extraction, PCR inhibition, competition with a strong positive BVDV signal, or will occur in cases where the intype IC-RNA had not been added to the lysis buffer prior to sample extraction.
Higher C_T-values in the Cy5 channel of a sample compared to most samples may indicate partial inhibition in the sample.

The **lack of a HEX fluorescence signal** in presence of a signal in the Cy5 channel indicates poor sample quality and/ or sample amount.

Table 4. Results interpretation table*

Sample result	FAM (BVDV)	HEX (EC)	*optional:* Cy5 (IC-RNA)	Interpretation
BVDV positive	X	X	X	Valid
BVDV positive	X	X		Valid (extraction without intype IC-RNA)
BVDV strong positive	X	(X)	(X)	Valid (no EC and/ or IC signal due to competition)
BVDV negative		X	X	Valid
Poor sample quality		X	X	Weak EC signal due to poor sample quality; recommendation to test a new sample
Partial PCR inhibition		X	(X)	No or weak C_T value for IC-RNA (recommendation to test 1:5 dilution of the sample)
Inconclusive			(X)	No signal for both EC and IC possibly due to failure during extraction or PCR

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The Positive Control must yield a signal in the FAM, HEX, and Cy5 channels. The Negative Control must yield no signal in any channel. For a complete explanation of possible sample results please refer to "Data analysis and interpretation" on page 16.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about afosa, bactotype, cador, cattletype, flocktype, pigtype, Svanovir and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

Notes

Notes

Notes

Limited License Agreement for virotype BVDV 2.0 RT-PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. INDICAL grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.indical.com. Some of these additional protocols have been provided by INDICAL users for INDICAL users. These protocols have not been thoroughly tested or optimized by INDICAL. INDICAL neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, INDICAL makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. INDICAL specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. INDICAL may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.indical.com.

Trademarks: afosa®, bactotype®, cador®, cattletype®, flocktype®, pigtype®, Svanovir®, virotype® (INDICAL BIOSCIENCE GmbH); ABI PRISM® (Applied Biosystems); Bio-Rad™ (Bio-Rad Laboratories, Inc.); FAM™, HEX™, JOE™, ROX™ (Life Technologies Corporation); Cy® (GE-Healthcare); Eppendorf® (Eppendorf-Netheler-Hinz GmbH). Licensed probes manufactured by Integrated DNA Technologies, Inc. Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

HB-2575-EN-002 © 2022 INDICAL BIOSCIENCE GmbH, all rights reserved.

Change index

Handbook	Version	Change
HB-2575-EN-002	July 2022	Editorial changes
HB-2575-EN-001	March 2022	Product launch

INDICAL
BIOSCIENCE

Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**